# Field Test for Repellency of Cedarwood Oil and Cedrol to Little Fire Ants

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**Abstract.** Eastern redcedars (*Juniperus virginiana* L.) are an abundant renewable resource and represent a potential source of valuable natural products that may serve as natural biocides. The aromatic wood can be extracted to obtain cedarwood oil (CWO) and critical carbon dioxide ($CO_2$) extraction of eastern redcedars gives both high yields and high quality CWO. In this study, $CO_2$-derived CWO and cedrol, the most abundant component of CWO, were field-tested for repellency against the little fire ant (LFA), *Wasmannia auropunctata* Roger, in a Hawaiian macadamia orchard. Field tests were conducted using chopsticks baited with peanut-butter placed in established LFA trails on macadamia tree trunks and branches. The chopsticks and any ants present were collected after ca. 24 hours and the number of ants determined by visual counting. Four treatments were compared: Hexane only control; mineral oil; CWO; and cedrol. Control chopsticks and chopsticks treated with mineral oil had very high numbers of ants and were statistically equivalent. The CWO-treated chopsticks had significantly fewer LFAs than all the other treatments. Chopsticks treated with cedrol had fewer ants than the control chopsticks but more than the chopsticks treated with CWO. This research suggests that CWO extracts from *J. virginianna* may provide a renewable source of a natural ant repellent and could help manage this invasive pest.

**Key words:** cedarwood oil, *Juniperus virginiana* L., cedrol, little fire ant, *Wasmannia auropunctata* Roger.

Eastern redcedar (*Juniperus virginiana* L.) (Cupressaceae) is widely distributed in the continental United States (Folwells 1965) and its range has been expanding recently (Ganguli et al. 2008). It is often considered a pest species because of its invasive character and its encroachment onto rangeland (Alemayehu et al. 1998). Eastern redcedar wood is well known for its aromatic smell and is the usual source of U.S. cedarwood oil (CWO; CAS no. 8000-27-9) which is typically obtained by steam distillation. However, supercritical carbon dioxide ($CO_2$) has been demonstrated to give higher yields than steam distillation as well as CWO with an odor more similar to the original wood and a much higher concentration of cedrol

(CAS no. 77-53-2) (Eller and King 2000). Cedrol, a sesquiterpene alcohol, is the most abundant component of $CO_2$-derived CWO (Eller and King 2000).

*Juniperus virginiana* mulch has been demonstrated to be repellent to several species of ants (Thorvilson and Rudd 2001, Meissner and Silverman 2001) and Anderson et al. (2002) reported a water suspension from *Juniperus* wood was repellent to red imported fire ants, *Solenopsis invicta* Buren (Hymenoptera: Formicidae). Recently, Eller et al. (2014) reported that CWO was repellent to *S. invicta* and prevented them from finding a typical food source (i.e., 10% sucrose solution). Also, "in an outdoor bioassay in Illinois, several species of ants were significantly repelled by the presence of CWO on a pole leading to a sugar-water solution" (Eller et al. 2014). Cedrol has also been demonstrated to have significant repellency towards *S. invicta*, although slightly less than CWO (Eller et al. 2014). Other similar sesquiterpenes (i.e., callicarpenal and intermedeol) from beautyberry (*Callicarpa* spp.) have also been shown to be repellent to *S. invicta* (Chen et al. 2008).

Another economically important ant, the little fire ant (LFA), *Wasmannia auropunctata* Roger (Hymenoptera: Formicidae), is originally from South and Central America and it is amongst the worst invasive ant species due the threats it posses to biodiversity, human health, and agriculture (McGlynn 1999, Conant 2000). It is listed amongst the "one hundred of the world's worst alien species" tabulated by the Invasive Species Specialists Group of the International Union for Conservation of Nature (Lowe et al. 2000). Ecological impacts include the displacement and reduction of native arthropods (Ulloa-Chacón et al. 1991), as well as stressing vertebrates and potentially causing blindness (Wetterer 1997, Wetterer et al. 1999). Besides administering painful venomous stings, LFA also impact agriculture by tending homopterans, which results in direct damage to crops and may vector diseases (de Souza et al. 1998). Motoki et al. (2013) discussed several means to manage LFA including residual pesticides which could form a "chemical barrier" to exclude LFA. Previous research on CWO suggests it could serve as a chemical barrier to LFA.

The objective of this research was to determine if CWO and/or cedrol are repellent to LFA in a field bioassay. If so, this could lead to a potential new management tool for the LFA.

## Materials and Methods

**Chemicals for repellency testing.** An eastern redcedar tree was harvested locally (Tazwell Co., IL) and heartwood sawdust was prepared as described by Eller et al. (2014). Cedarwood oil was extracted from this sawdust using supercritical carbon dioxide (70°C; 4000 psi) as described by Eller and King (2000). The CWO composition was determined by gas chromatography (relative peak areas) (Eller and Taylor, 2004). The (+)-cedrol was purchased from Aldrich (Milwaukee, WI). The mineral oil (CAS no. 8042-47-5) and HPLC grade hexane (CAS no. 110-54-3) were purchased from Fisher Scientific (Pittsburgh, PA).

**Field repellency bioassay.** There were four treatments tested: hexane control (100 µL); mineral oil (50 µL); CWO (50 µL); and cedrol (100 µL of a 175 µg per µL solution in hexane, i.e., 17.5 mg). This amount of cedrol approximated the amount of cedrol present in 50 µL CWO. The test materials were applied to the middle section of a wooden chopstick (ca. 4 mm wide by 20 cm long) (Island Accents, CVS Pharmacy). The field test was conducted in a commercial macadamia nut orchard known to have LFAs near Papaikou,

**Figure 1.** Treated chopstick placement on a macadamia tree branch.

**Figure 2.** Little fire ants travelling up and down a control chopstick.

Hawaii (19.787029, –155.124443). The test trees were ca. 5 meters apart within a row and there was ca. 7 meters between the rows. Macadamia trees with active LFA trails were located and subsequently all four treatments were attached to each of these trees (Figure 1). The treated wooden chopsticks were placed in the ant trail and attached to the tree using pins ca. 10 cm apart from one another. The order of the treatments on each tree was randomized. Approximately 1 mL of peanut butter (Skippy® Creamy, Hormel Foods Corp., Austin, MN) was applied to the top the chopstick to serve as an attractant food source (Starr et al. 2008, Hara et al. 2014). Foraging ants found these sticks and traversed up and down the sticks (Figure 2). After ca. 24 hours, each chopstick was carefully collected, placed into a plastic bag and sealed. The sealed bags were transported to the laboratory and placed in a freezer to kill ants present. Subsequently, the number of ants on each chopstick was determined by visual count.

Field bioassays were conducted over three consecutive days (August 10–12, 2015). There were 20 replications (i.e., trees with each of the four treatments) on the first day and 30 replications on each of the second and third days. Trees were only tested on one day and not reused.

**Statistical analyses.** A 2-factor mixed effects hierarchical design nested model analysis of variance (ANOVA) was used to analyze LFA count data differences between the four treatments over 3 days (SAS version 9.3 @ 2002-2110 [SAS Institute Inc., Cary, NC]). Different trees were used for each day so the nesting factor was tree nested within day. Levene's homogeneity of variance (HOV) test was performed to test for data transformation necessity. Levene's HOV test was applied after trees with treatments having zero mean and variance were removed as well as those identified as outliers from Box plot analyses. The cube root (number of ants) transformation stabilized the variance so that ANOVA assumptions were

**Table 1.** Day by treatment interaction ant count means using the SLICE option in SAS to compare ant counts between treatments at each day.

| | Treatment[1] | | | | |
|---|---|---|---|---|---|
| **Day** | **Control** | **Mineral oil** | **Cedrol** | **Cedarwood oil** | **P-value[2]** |
| 1 | 41.3 a | 39.2 a | 35.4 a | 2.0 b | <0.0001 |
| 2 | 176.8 a | 193.4 a | 109.8 b | 22.2 c | <0.0001 |
| 3 | 23.5 a | 24.7 ab | 10.5 b | 2.1 c | <0.0001 |

[1]Ant count treatment means at each day (within a row) without letters in common differ significantly based on differences of least squares means at $P \leq 0.05$.
[2]P-values for treatment mean differences in ant counts at each day from SLICE option on day by treatment interactions.

met. Because the F-test statistic for the day by treatment interaction was significant ($P<0.0001$), the SLICE option in SAS was used to examine treatment differences at each day as well as day differences for each treatment. All analyses were performed on transformed values where necessary, but untransformed data is presented for ease of interpretation. Outliers were removed after identification from box plots for each treatment on each day (JMP version 11.2). Trees having any treatments with zero mean and zero variance were also removed from the analysis.

## Results and Discussion

**Field repellency bioassay.** Table 1 shows the mean number of ants for each treatment by day combination. The numbers of ants were clearly affected by the day and the day effects were undoubtedly a result of the weather conditions. Day 1 had some rainfall; day 2 was a very pleasant day, mostly sunny without rain; while on day 3, tropical depression Hilda had arrived to Hawaii and brought with it heavy rainfall. The heavy rainfall appeared to slow the foraging activity of the LFA and subsequent captures on the chopsticks.

The control and mineral oil treatments had the highest numbers of ants and were statistically equivalent for all three days. The CWO treatment had the lowest number of ants and was significantly lower than all the other three treatments on all three days. The cedrol treatment was somewhat non-preferred as it had significantly fewer ants than the control on two of the three days; however, it was statistically equivalent to the mineral oil treatment on two of the three days.

The cedrol treatment was not optimally formulated for this experiment. Crystals of the cedrol formed on the chopstick and it therefore did not form an even chemical layer completely around the chopstick (Figure 3). Ants were observed working their way through the cedrol crystals to and from the peanut butter bait. Although the addition of 10% mineral oil to the cedrol:hexane solution appeared to decrease the crystallization of the cedrol (i.e., smaller crystals were formed), a relatively high number of ants were still found on the cedrol-treated chopsticks. A different formulation of cedrol may have resulted in greater activity for cedrol. A solvent that evaporated much more slowly

**Figure 3.** Cedrol crystals formed on chopstick.

than hexane may have provided a more even distribution of cedrol around the chopstick. After these field trials were completed, it was discovered that cedrol can be dissolved in neat mineral oil and this solution does not form crystals after application. This could be a suitable means to formulate cedrol for future studies. CWO is a mixture of over 30 compounds which contains cedrol as well as many other compounds including thujopsene (CAS no. 470-40-6) and α-cedrene (CAS no. 469-61-4) (Heide et al. 1988; Adams, 1991). Although isolated cedrol is a solid at room temperature, it is part of a stable solution within the complex CWO mixture which typically contains high levels of the liquids thujopsene and α-cedrene. This CWO mixture allows the formation of an even coating around the chopstick.

The observed lower activity of cedrol relative to the CWO may indicate that other compounds in the CWO are responsible for CWO's higher activity. Previously, cedrol has been shown to be repellent to the *S. invicta*, however, cedrol alone did not account for all of the repellency observed for the CWO mixture (Eller et al. 2014). It is likely the higher activity observed for CWO against both *S. invicta* and LFA is a result of several components in CWO. Although cedrol is the most abundant component in $CO_2$-derived CWO (Eller and King 2000) and it even if cedrol was determined to be the most active component in the CWO, it might be more cost effective to use the CWO mixture rather than isolated cedrol.

Similarly, although juniper mulches have been demonstrated to be repellent to several species of ants (Thorvilson and Rudd 2001, Meissner and Silverman 2001), the relative effectiveness of these mulches versus extracted CWO is unknown. The importation of eastern redcedar mulch into Hawaii could be problematic. On the other hand, CWO might be more convenient because it could be formulated like other liquid pesticides and used to treat affected areas.

## Conclusions

These results demonstrate that $CO_2$-derived CWO extracts from *J. virginiana* can significantly reduce the number of LFA utilizing a preferred food source. Although our tests only were conducted over a 24-hour period, because CWO is not volatile, it would not evaporate quickly and could be expected to be active much longer than our 24-hour test. Cedarwood oil is Generally Recognized As Safe (i.e., GRAS) as defined by the U.S. Food and Drug Administration, and the U.S. Environmental Protection Agency has exempted CWO from federal pesticide regulation because CWO poses little or no risk to public health or the environment (U.S. Food and Drug Administration, 2013). Therefore, CWO could become a safe natural alternative to other ant repellents, as well as being a renewable product from invasive cedars and cedarwood wastes (i.e., co-product sawdust) not currently utilized (Adams et al. 1988). The demonstrated bioactivity of CWO suggest that CWO could serve as a mitigation treatment as part of a larger management program against the LFA.

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## Disclaimer

Mention of trade names or commercial products in this article is solely for the purpose of providing scientific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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